CXXVII. THE COLORIMETRIC DETERMINATION OF RHAMNOSE.

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THE estimation of the methylpentoses, of which rhamnose is the best known, is usually carried out by distilling the material for analysis with 12 % HCl. Methylfurfuraldehyde is formed and can be estimated in the distillate gravimetrically, or by one of the methods based upon its reducing power [Pervier and Gortner, 1923]. These distillation methods require much time and attention and are essentially macro-methods.

The micro-method now to be described is accurate; it is also rapid because large numbers of estimations can be carried out simultaneously. The principle is similar to that of the method already described for the estimation of pentoses [McCance, 1926].

Methylfurfuraldehyde (unlike furfuraldehyde) does not form a coloured compound with aniline or benzidine, both of which have been used for the colorimetric estimation of the simple pentoses such as xylose and arabinose [Youngburg and Pucher, 1924; Hoffman, 1927]. With phloroglucinol, however, methylfurfuraldehyde forms a soluble coloured compound which has been adapted to the estimation of rhamnose.

Solutions and reagents required.

- (1) Pure concentrated HCl.
- (2) Benzene.
- (3) 0.25 % phloroglucinol in 98 % alcohol.
- (4) 0.1 % solution of rhamnose (standard).

Procedure.

3 cc. standard, 3 cc. water and 3 cc. concentrated HCl are mixed in a test-tube. An amount of the unknown solution containing about 3 mg. of rhamnose is put in a second test-tube. Water is added to 6 cc. and finally 3 cc. concentrated HCl. The whole is mixed. Unknown and standard are then placed in a water-bath at 100° for 2 hours, each with a cork fitted with a long glass

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tube as an air condenser. After removal from the water-bath and cooling, 4 cc. of benzene are added both to the unknown and to the standard and the test-tubes are vigorously shaken for at least 3 minutes. They are then allowed to stand for 20 minutes. By spinning between the fingers and by lifting the tube an inch and allowing it to drop back into its place in the rack the benzene layer above the unknown can be readily cleared of black "humic" matter which may appear in it after shaking. 2 cc. of the supernatant benzene of the unknown and standard are then removed with a pipette and placed in clean dry test-tubes. 4 cc. of phloroglucinol solution are added to each and finally, with shaking, 0.65 cc. of concentrated HCl. A deep yellow colour at once appears which continues to develop for about 3 minutes. If the unknown solution is urine, the colour match should be made between 3.5 and 4 minutes after adding the HCl, for something is extracted by the benzene from urine under these conditions which slowly develops a colour with phloroglucinol indistinguishable from that of the methylfurfuraldehyde compound. This does not affect estimations carried out 4 minutes after adding the HCl. The colour of the standard remains unchanged for at least 0.5 to 1 hour so that a large number of separate unknowns may be estimated in succession against one standard, each being matched 3.5–4 minutes after adding the HCl. The reading of the unknown should not be far removed from that of the standard if accuracy is desired.

Scope and limitations of the method.

(1) The following figures illustrate some of the recoveries which have been obtained by this method when known amounts of rhamnose have been added to a urine. (The solutions were made up by one and estimated by a second person.)

Rhamnose added (mg. per 100 cc. urine)	Rhamnose recovered (mg. per 100 cc. urine)
200	200
100	99
67	65
50	54

(2) When hexoses are boiled with HCl, small amounts of hydroxymethylfurfuraldehyde are formed in amounts varying with the hexose. These substances react with phloroglucinol giving a yellow colour similar to that produced by methylfurfuraldehyde. The error introduced into the estimation of rhamnose by the presence of amounts of hexose equal to the rhamnose has been determined and found to be: with fructose 3.0 %; with glucose 1.4 %; with mannose 2.0 %; with galactose 0.35 %.

(3) The presence of other pentoses (arabinose or xylose) interferes with this method as phloroglucinol forms a deep blackish green compound with furfuraldehyde.

SUMMARY.

A colorimetric method of estimating rhamnose is described, based upon the reaction between phloroglucinol and methylfurfuraldehyde. The methylfurfuraldehyde formed by heating rhamnose with hydrochloric acid is separated, for estimation, by extraction with benzene and not by distillation.

REFERENCES.

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